CHAPTER V

NITROGENOUS EXCRETION

THE DIET of any animal contains a multitude of organic compounds, carbohydrates, fats, amines and proteins among them. From these the animal builds its own structure and obtains energy for the many enzyme systems necessary to it. The protein of the cells and secretions is manufactured from the amines formed by hydrolysis and degradation of the proteins in the food. These are absorbed, transported to the sites utilizing them and the characteristic proteins of the body are then formed. This is not the end of the story, however, for as was shown by the classic studies of Schoenheimer and Rittenberg the body proteins are in a continual state of flux, changing constantly. The materials no longer required are removed, broken down to non-toxic units and excreted. In some cases they may be excreted little altered e.g. creatinine from creatine in vertebrates, but in the majority of cases the nitrogen fragment is converted to a non-toxic substance and removed in the urine. If the animal has access to voluminous quantities of water ammonia is the most common and most easily voided excretory product e.g. in amphibians, fish and marine invertebrates. In land-dwelling species water conservation is of great importance and the production of small quantities of urine containing high concentrations of nitrogenous materials is the rule. The less toxic products urea and uric acid make their appearance in these forms.

Nitrogen is also lost in many cases in the form of muco-proteins, secreted by epidermal cells on to the surface of the body for the purposes of lubrication, maintaining a clean body surface and providing a buffer against the external environment. Oligochaetes are no exception to these generalizations, and once again the vast majority of the published work has been concerned with earthworms.

One of the earliest attempts to ascertain the excretory products of earthworms was made by Lesser (1908) on *L. terrestris* and *E. foetida*. Using the limited techniques available in those days he found no evidence for the occurrence of uric acid, urea or allantoin, but did obtain results indicating the presence of ammonia. Much later Florkin and Duchateau (1943) showed that this was not altogether surprising for earthworms possess no allantoinase, allantoicase or uricase and cannot hydrolyse allantoin, allantoic acid or uric acid respectively. These authors did find that xanthine oxidase, the uric acid forming enzyme, is apparently available. Urease has been reported by Przlecki (1923) to break down urea and form ammonia at the very slow rate of 2·28 mg ammonia/100 g/day. This may have been erroneous, however, and perhaps due to bacterial action for Abdel-Fattah (1957) found no signs of urease activity in blood, coelomic fluid, body wall or gut of *L. terrestris* or *A. longa*, although ammonia and urea were both to be found in these tissues.

The nitrogenous excretion of earthworms may be considered as occurring in two fractions, each accounting for approximately equal portions of nitrogen removed each day. The first portion consists of a protein amounting to about 0·030 g/100 ml urine/day (Bahl, 1947a, b), and is most probably derived from the mucus secreted copiously by the body wall. This mucus acts as a lubricant as the worm proceeds along its burrow, also helping to bind soil particles together and preventing the burrow walls from collapsing. Mucus also probably acts as a buffer system outside the body since it is secreted in large amounts when the animal is immersed in a noxious stimulant such as acid. This mucoid protein can account for about half the total nitrogen lost each day (Needham, 1957; Haggag and El-Duweini, 1959), though this may be a reflection of the somewhat unnatural conditions encountered by the animals in experimental vessels.

The second fraction, representing the end products of metabolism, is a fluid urine comprising a mixture of ammonia and urea, with rather confused reports also on the presence of uric acid and allantoin (Fig. 16). The proportions and presence of these nitrogenous substances depends upon the species and upon the condition of the animals when examined.

Lesser (1908) and Delaunay (1934) both isolated earthworms

into large volumes of water for varying periods of time, and then analysed the resulting fluid. As we have mentioned above Lesser had difficulty in deciding what nitrogenous substances are present, being certain only of ammonia. Delaunay (1934) found that ammonia is the largest single excretory product detectable in the urine, and this is allied to urea, and a little amino nitrogen. He found no uric acid. Under the conditions of the experiments, however, considerable dilution of the urine occurred and no reliable

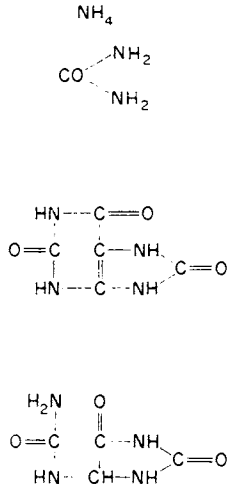

FIG. 16. Formulae of ammonia, urea, uric acid and allantoin, the presence of which have been reported in the urine of earthworms.

estimate can be made as to the volume and chemical concentration of the urine produced. More recently results on urine composition have been obtained from earthworms placed in small volumes of water (Needham, 1957), in clean flasks washed out periodically (Cohen and Lewis, 1949b), in moist air with urine allowed to drain away down a slope (Bahl, 1947a) and by collection of urine direct from the nephridiopores (Ramsay, 1949a). Unfortunately the last-named method, by far the most accurate technique, has been used so far only for estimating the osmotic pressure and mineral salt content of the urine, and the concentration of

nitrogenous compounds has not been determined in the small quantities available by this micropipette method.

The losses to evaporation and dilution by excess fluid are, however, minimal in the experiments of Cohen and Lewis (1949 a, b) and Needham (1957) but somewhat conflicting results have been reported by these workers. Normal, healthy, fed *L. terrestris* excrete an average of 5·75 μg NH_3/g wet wt./day, which represents approximately 70–80% of the total non-protein nitrogen. Urea accounts for about 8% of the total (0·37 μg/g wet wt./day): Cohen and Lewis (1949 a, b) record 0·16 μg/g wet wt./day allantoin and 0·113 μg/g wet wt./day uric acid, substances not found by some other authors. A period of starvation is followed by an inversion in excretion of the major constituents, ammonia falling to 1·8 μg/g wet wt./day, whilst the urea fraction rises to 18·15 μg/g wet wt./day. At the same time the total amount of non-protein N excreted rose from 7·4 μg/g wet wt./day to 21·5 μg/g wet wt./day.

Very different absolute values were obtained, however, for the same species, by Needham (1957). Estimates of the total non-protein nitrogen content of urine in animals feeding on elm leaves amounts to 94·8 μg/g wet wt./day and starvation raises this level to 159·2 μg/g wet wt./day. The major components of nitrogenous excretion are confirmed as ammonia (54 μg NH_3/g wet wt./day) and urea (40·8 μg/g wet wt./day). Starvation of the animals alters the relative importance of these two substances, the output of urea rising by some 300% whilst ammonia decreases 30%. Thus although ammonia is present in excess of urea in normal feeding earthworms and the changes seen upon starvation are in the same direction as those noted by Cohen and Lewis (1949b), they differ considerably in the ratios of NH_3 : urea, and in absolute values by a factor of approximately 10 (Table 7).

In his 1957 paper Needham reports on observations made on two species of earthworms, *L. terrestris* and *E. foetida*. These two species are fairly closely related but show dissimilar patterns of nitrogen excretion. Table 7 compares the nitrogen output of these two species and reveals that whilst the urea production of the two forms is closely similar there is a considerable discrepancy between the ammonia released by each species, that of *E. foetida* being double that of the much larger *L. terrestris*. Needham finds no evidence that the roles of urea and ammonia are interchanged in the

TABLE 7

LEVELS OF EXCRETORY SUBSTANCES REPRESENTED IN THE URINE OF VARIOUS EARTHWORMS

Species	*L. terrestris*		*L. terrestris*		*E. foetida*		*A. caliginosa*		*Pheretima*
	B	A	B	A	B	A	B	A	
Urea	0·37	18·15	40·8	129·1	48·3	73·1	19·9	83·2	3·24
NH_3	5·75	1·85	54	30·1	104·85	228·8			2·66 mg/100 ml
Amino N					51·7	43·6	37·4	14·0	
Allantoin	0·16								
Uric acid	0·113		1·42						
Total non-protein N	7·4	21·5	94·8	159·2	204·8	345·5	87·5	133	
Author	Cohen and Lewis, 1949		Needham, 1957		Needham, 1957		Haggag and El-Duweini, 1955		Bahl, 1947b

B = Before starvation
A = After starvation in μg/g/day

excretory picture of *E. foetida* after a period of starvation, both bear the same relationship to one another although the actual quantities involved are doubled. *L. terrestris* on the other hand shows a trebled output of urea after inanition, but a slight drop in the ammonia production. *A. caliginosa*, another small species, shows a nitrogen excretion of similar pattern to *L. terrestris* though it does not excrete such large quantities of urea or ammonia.

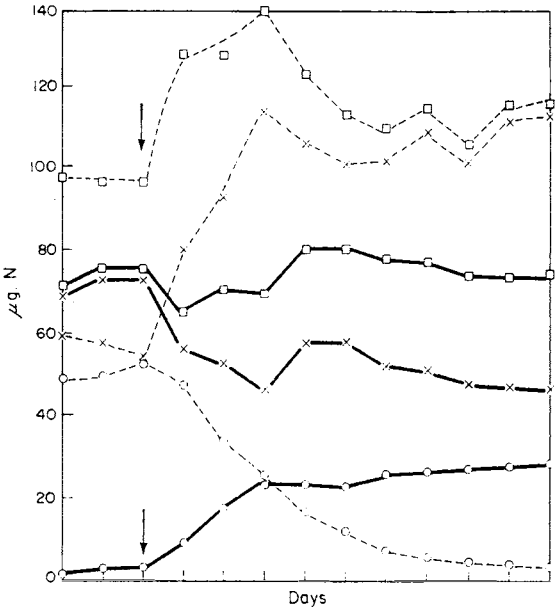

FIG. 17. Excretion of urea N(×) amino+ammonia-N(○) and amino+ammonia+urea-N (□) *Lumbricus* during transition from feeding to fasting (- - - - -) and from fasting to feeding on elm leaf (——) in μg/g body wt./day (from Needham, 1957).

Fig. 17 shows the course of excretion over a period of days in *Lumbricus*.

Uric acid accounts for only a very small percentage (1·5%) of the total nitrogen excreted in fed *L. terrestris* and a rather larger proportion in *E. foetida* (3·4% in fed animals, 3·35% in starved individuals). In this work no mention is made of allantoin. When the posterior region of the animal is removed the excised portion

excretes nitrogen at a high rate, mainly as protein, until the piece dies.

Urine as excreted from the earthworm body is acidic. The acidity is shown to vary from time to time and starving animals produce less titratable acid than do feeding worms. *E. foetida* produces more acid than does *L. terrestris*, but whereas the pH of the urine is buffered by urea in the case of *L. terrestris*, as shown by a rise in pH upon treatment with urease, this is not so in *E. foetida*. Needham (1957) suggests that in the latter case the buffering of the urine is a function of some non-nitrogenous akali, citing as a possible mechanism the calcium carbonate removed from the calciferous glands.

Thus in these two related species the excretory products differ in a number of ways. *E. foetida* has a much higher specific output of total nitrogen and of the ammonia fraction than does *L. terrestris* and ammonia makes up a larger part of the total excreted. The ammonia excreted does not go down, but rather goes up, when the animal is subjected to fasting. *E. foetida* has a higher removal of acid, and it is believed utilizes a non-nitrogenous base to neutralize it, possibly the calcium carbonate from the calciferous glands, which is thought to be playing a part in the internal regulation of pH as well (Voigt, 1933). In the conditions of Needham's experiments the anal and nephridial excreta were able to mix freely, and thus his explanation might be true for this set of circumstances, but in the field the animal is free ranging in a burrow, and as it is probable that urine from the nephridia is immediately absorbed on to soil particles as it is released, it seems unlikely that concretions issuing from a distant site will have much effect upon the urine pH.

Cohen and Lewis (1949b) and Needham (1957) provide clear demonstrations as to variations in excretory products of earthworms when they are starved, although they do not agree on the amounts involved. That the geographical location may be important in arriving at results and conclusions seems to be indicated by the work of Haggag and El-Duweini (1959). *A. caliginosa* excretes nitrogen in a pattern similar to that of *L. terrestris* (Needham, 1957) though the absolute amounts are much smaller. In Egyptian representatives of this species protein accounts for up to 50% of the total nitrogen excreted, similar to that reported by Needham.

Unfortunately Haggag and El-Duweini (1959) do not give details of the nitrogenous content of urine in conditions of no starvation, but figures for excretory values for periods of up to 27 days without food reveal that no systematic changes in the proportion of ammonia or urea occur. In animals starved for 5 days the ammonia fraction accounts for 54·2% of the total excreted nitrogen, whilst urea amounts to 6·25% and uric acid 0·26%. No indication, however, is given of the actual quantities involved. Prolonged inanition leads to alterations in the amounts of these substances represented in the urine but in no case does the urea content exceed that of ammonia. This is in direct contrast to the results of Needham (1957) on the same species in England.

The availability of water in the environment may obviously vary considerably from time to time and such variations may be of great importance in the excretory mechanisms utilized by oligochaetes. The change to a terrestrial habitat made by many oligochaetes means that water is always at a premium. We shall see later that the earthworm is rather like a fresh-water animal in its water relationships. In animals living in a great excess of water the more highly soluble nitrogenous products such as ammonia and trimethylamine oxide are very often the sole excretory products, but with the changeover to the land and a decreasing volume of available water first urea and then uric acid becomes the dominant substance, each less toxic than the preceding one.

The earthworm hasn't settled upon one excretory substance to do the job, relying mainly on ammonia, which is removed into the soil moisture usually found in excess around the body, but also excreting urea. The amount of the latter that is excreted seems to depend upon the metabolic water available since starvation leads to a decrease in ammonia and an increase in urea content of the urine. Needham (1957) immersed earthworms in varying quantities of water, for varying periods of time, and concluded that the volume of water was unimportant unless accumulation of toxic products was allowed to occur over a long period. Again this is unlikely to occur in the field. No experiments have so far been carried out on the effect of dehydration upon the excretion of these animals, a state of affairs just as likely to happen in nature, but obviously involving technical difficulties in the collection of adequate samples for analysis. The nephridiopore collection method of

Ramsay (1949 a, b) might have a useful application here though the quantities obtained would be extremely small.

Although no information can be presented regarding the changes of urine content, if any, during desiccation it is possible to show that the tissue content of nitrogenous substances changes its nature during such conditions. Analysis of tissue homogenates from fresh *A. caliginosa* show that ammonia nitrogen amounts to 26·48 mg/g tissue nitrogen, urea 13·14 mg/g N and uric acid 1·33 mg/g N. These values, like those for urine content, do not change during starvation (in the experiments of Haggag and El-Duweini, 1959). When the animals are allowed to dry in air, however, the ammonia and uric acid content remain quite stable but urea values rise considerably (Table 7).

This rise in urea content can be correlated with a gradual decrease in the amount of water present suggesting that the earthworm is able to adapt its excretory pattern in times of internal water stress by carrying the metabolic cycle further to produce urea instead of ammonia. The production of the even less toxic uric acid, however, is not affected, perhaps due to a limited enzymatic apparatus (xanthine oxidase) which is unable to deal with the increased conversion required (Florkin and Duchateau, 1943).

Haggag and El-Duweini (1959) made no differentiation between the various organs of the body, homogenizing the animals entire without previous separation of the parts. That there is a partition of ammonia and urea between the gut and the body wall has been shown by Heidermanns (1937) for *L. terrestris* and by Bahl (1947b) for *Pheretima posthuma*. Analysis shows that the gut wall contains more urea than the body musculature, and also more ammonia, though the differences here are not so pronounced (Table 8).

Creatinine was reported by Bahl (1947a) as a breakdown product in both body wall musculature and intestine in *P. posthuma*, and Abdel-Fattah (1957) obtained a figure of 1·60 mg% for the creatinine content of urine from *Lumbricus* and *Allolobophora*. The methods used in these estimations are not, however, specific and with the discovery of lombricine in oligochaetes, and other guanidine derivatives in sundry other annelids it is highly likely that the so-called creatinine fraction is in fact some other guanidine product.

From these analyses it is apparent that urea and ammonia occur as metabolic products in the tissues, notably the intestine, passing from there into the blood or coelom and from thence to the exterior via the nephridia. The ability of the intestinal wall to form urea was first shown by Heidermanns (1937) who incubated homogenates of this organ with "peptone" and obtained urea, this substance not being formed when homogenates of the body wall were incubated with the same substrate. The provision of arginine or ornithine as substrate had no effect upon the elaboration of urea and it was concluded that urea is not formed by the Krebs–Henseleit series of reactions.

TABLE 8

NITROGENOUS CONTENT OF TISSUES OF TWO EARTHWORMS
(values mg%)

	P. posthuma		*L. terrestris*	
	gut	body	gut	body
NH_3	4·3	3·6	7·7	3·3
Urea	4·6	2·1	15·3	1·6
Creatinine	4·4	5·5		
Author	Bahl, 1947b		Heidermanns, 1937	

Heidermanns used simple water extracts in his work, and repeating his observations Cohen and Lewis (1950) were also unable to obtain a yield of urea when intestinal preparations of *L. terrestris* were incubated with arginine. They were also unable to find urea after incubation with "peptone", a result in direct contrast to that of Heidermanns, and for which they were unable to account. However, using tissue homogenates in phosphate buffer solutions, arginase activity is found (Cohen and Lewis, 1950) when the intestine alone is considered, but if the whole body is macerated and incubated then no arginase is observed. The optimal pH for the enzyme is pH 7·2–8·6, and a co-factor in the form of 10^{-3}M cobalt increases the activity. Fresh captured and recently fed animals are found to possess only slight arginase

activity, but the concentration increases by a factor of 10 when animals are starved for up to 4 weeks. This change in enzyme activity can be correlated with the previously described alteration in the importance of urea as a metabolic product during inanition.

Cohen and Lewis (1950) also utilized an injection technique to study the method of formation of urea within the earthworm. They inserted a fine hypodermic syringe into the gizzard of anaesthetized animals and injected amine compounds such as ornithine, arginine, citrulline, glycine, glutamine, alanine, histidine and hydantoin into the gut. The excretion of urea is affected only by arginine and citrulline when used alone. But if citrulline is injected simultaneously with another source of nitrogen such as alanine or glutamic acid, urea is formed in even greater amounts.

Therefore, although Heidermanns found no evidence for the presumption that the ornithine cycle is active in earthworms it now appears that such a cycle does in fact exist in these animals. Citrulline and arginine are both intermediates in this system, and arginase is the enzyme necessary for the breakdown of arginine to form urea and ornithine. These three steps now seem fairly well established, and further evidence on this point will be presented later. The observation that the major part of the arginase activity is located in the intestinal wall rather than the body wall is suggestive of the role of chloragogen cells in excretion. Heidermanns (1937) termed the chloragogen tissue "the central organ of urea metabolism". Needham (1960) has provided confirmation of the presence of arginase in *Lumbricus terrestris* and *E. foetida* tissues, and in the greater activity of gut preparations in this respect compared with body wall preparations. The activity of the enzyme varies in direct relationship to the urea excreted under feeding and fasting conditions.

The Chloragogen Tissue

The function of the chloragogen tissue in the metabolic economy of the earthworm has been a vexed question for many years. Opinion has wavered between two extremes. First, it has been argued that the position of these cells, closely applied to the coelomic surface of the intestinal wall, is ideal for the development of a liver-like function. Food substances absorbed through the gut wall can be transferred into the chloragocytes, which by

migration may transport the nutrients through the body or which may release these substances into the blood stream or coelomic fluid, controlling the amount in circulation by homeostatic means.

The second theory holds that these cells are excretory in function gathering waste products, which are presumably circulating freely in the blood, or coelomic fluid, concentrating them, transforming them when necessary into urea, or ammonia and then the cells detach from the gut wall to autolyse in the coelom from whence the fluid is voided via the nephridia. As high levels of urea and ammonia have been reported for both blood and coelomic fluid it is

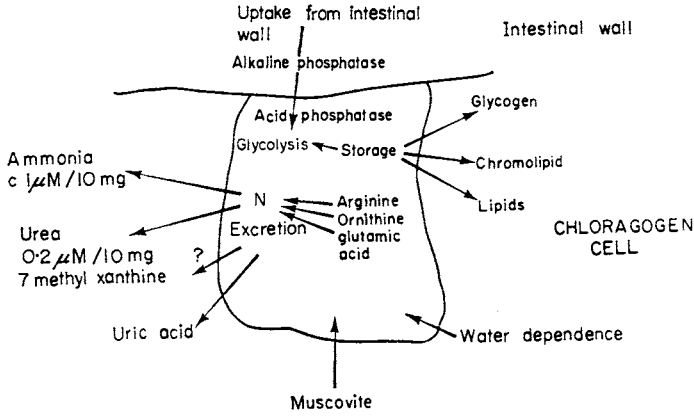

FIG. 18. Role of chloragogen cell in metabolic relations of the earthworm.

possible that both fluid systems serve to bring waste products to the nephridia, one from the tissues by means of blood vessels, and the other from the internal coelom. In earthworms with nephridia that are closed internally, of course, it is not possible for the coelomic fluid to play a part in excretion by simple filtration, only by bathing the nephridia so that diffusion can occur across the nephridial wall into the lumen.

What actually happens in the chloragocytes is probably a mixture of both the above points of view as indicated by the work of van Gansen (1956, 1957b, 1958) and Roots (1957, 1960) (Fig. 18) with which we shall be mainly concerned here, although Liebmann

(1942 a, b) has much interesting work on the nutritive functions of chloragogen which is dealt with in the section on regeneration.

The presence of food substances such as glycogen and fat has been reported at intervals since early this century, but the most exhaustive study made so far is that of van Gansen (1956). The most important food reserve, if bulk be the criterion, is glycogen. In fresh feeding *A. caliginosa* a concentration of 62·3 μg/mg dry wt. of intestine is obtained, a quantity which decreases drastically to 33·6 μg/mg dry wt. after the animals have been starved for one month. Lipid substances, also possible energy stores, are found in two forms, lipids and fats, the former remaining unaffected by starvation, the latter being depleted. Alkaline phosphatase, an enzyme often found in rapidly metabolizing tissue, is not present, its place being taken by an acid phosphatase. The probability that the materials within the chloragocytes originate in the intestinal wall is suggested by the occurrence of large concentrations of alkaline phosphatase in the distal ends of the intestinal cells lying alongside the chloragocytes, indicating the active transport of absorbed substances across the cell boundaries of this region.

The chloragocytes contain many obvious granules. Those at the periphery of the cells stain intensively with Sudan black and histochemical methods (PAS, PFAS tests) show that phospholipids are present, and contain ethylene groups. The typical brown-yellow colour of the chloragocytes is imparted by a chromolipid, the alimentary origin of which is not immediately apparent, but which is possibly an oxidized phospholipid which polymerizes with purines within the cell (van Gansen, 1957b). Roots (1960) has discussed this chromolipid and shown that it consists of a phospholipid allied with a yellow-brown spectrally uncharacterized material which she thinks may indicate that the pigment is a very complex mixture of substances in equilibrium. Other inclusions within chloragocytes include mineral aggregates, identified in electron microscope and X-ray diffraction studies as muscovite or mica (van Gansen and van der Meersche, 1958). Roots (1960) finds that not all the inorganic ash of the cells is due to silica and believes that where this substance is present it is due to accidental penetration from the lumen of the gut. The siliceous granules are not to be found universally in the chloragogen.

The facts mentioned above are all in support of the theory that

chloragogen tissue is active in the metabolism of the animal, absorbing, transforming and mobilizing energy sources. But although the tissue is ideally placed for the absorption of material from the gut wall it is not easy to see how it is made available for the other tissues of the body. Three possible methods of distributing the contents of these cells are as follows: first, it is thought that chloragogen cells are able to leave the site of origin on the intestinal wall and to wander freely in the coelomic cavity. They are able to pass from segment to segment and can be accumulated at situations wherever necessary. For example it has been noted (Liebmann,

TABLE 9

NITROGENOUS COMPOUNDS PRESENT IN URINE, BLOOD AND COELOM OF THREE EARTHWORM SPECIES (μg/100 ml)

	P. posthuma			*Lumbricus* and *Allolobophora*		
NH_3	2·7	4·0	2·7	3·22	1·56	2·04
Urea	3·2	2·6	2·5	7·46	5·47	5·91
Creatinine	0·5	3·5	2·7	1·6	5·4	6·6
Protein	30·0	3640·0	480·0			
	Urine blood coelom Bahl, 1947b			Urine blood coelom Abdel-Fattah, 1957		

1942 a, b, 1946) that when a missing section of the body of an earthworm is regenerated the cicatrice area becomes packed with a mass of chloragogen tissue within a very few hours after wounding, and that these cells break down in the wound area and release their contents into the coelom. Presumably the actively proliferating cells repairing the scar and regenerating the new tissue are able to utilize the materials thus brought into the region.

Secondly, in unwounded animals the chloragogen cells also wander in the coelom, and as they do so they autolyse and the inclusions, glycogen, fats, etc., disappear into solution in the fluid. The bodily remnants are phagocytozed by amoebocytes. Quite what happens to the chemical substances thus released is as

yet untraced. Some facts are known about the concentrations of substances within the coelom (Table 9) but in general the higher molecular weight organic compounds such as glucose, proteins and amino-acids are at a very low concentration compared with the blood values of the same materials. On the other hand there is less discrepancy between the quantities of nitrogenous substances in blood and coelomic fluid. This may mean that the nutrient materials are speedily removed from solution by the tissues bathed by the coelomic fluid, and consequently the concentration of these substances always tends to be low. Alternatively the chemicals may be re-absorbed into the blood system which then carries the necessary raw materials to the tissues. The blood system then, is our third possibility as a transport mechanism. Since the intestinal wall is very well provided with a plexal system of blood vessels it is easy, but unproven, to visualize the likelihood of food substances being absorbed from the gut into the blood system and distributed by this system as it wends its way round the body. Perhaps the chloragogen is indeed liver-like, acting as a homeostatic device to maintain a constant level of circulating substances. It has the added advantage of being a mobile liver and able to migrate to areas of great need.

The excretory function of the chloragogen cells for a long while rested upon a report by Willem and Minne (1900) who considered that the granules contained within the cells are composed of guanine, an end product of nitrogenous metabolism. This has been brought into doubt by Peschen (1939), Abdel-Fattah (1955), van Gansen (1956) and Roots (1957). Roots (1957) doubted the importance of chloragogen tissue as a site for nitrogenous metabolism. She isolated the granules of the cells, and analysed them to find a composition of C, 43%; H_2, 6%; N, 4%; P, 3·5% and S, 1%; as guanine contains 60% N it cannot be present, and it seems unlikely that other nitrogen excretory compounds are to be found as granular inclusions in the cell. But although the more complex granules may not be present there is adequate evidence that other simpler nitrogen compounds are present, and these would have been overlooked in Roots' (1957) work, since she was not concerned with materials in the liquid phase. A report that guanine is also found in the walls of the nephridia has been discounted by Bahl (1947b).

The discrediting of guanine as an excretory product has been followed by the demonstration of another purine base in these cells. Van Gansen (1956, 1957) working on the chloragogen of *A. caliginosa* found that a purine derivative, having an absorption spectrum different from that of guanine, but similar to that of heteroxanthine, a product commonly found in mammalian urine, is present in the chloragogen (Fig. 19). Heteroxanthine, or 7-methyl-xanthine, may thus prove to be of importance in the removal of one class of chemical substance from the body. This suggestion has been criticized, however, by Abdel-Fattah (1955), and also by Roots (1960) who found no trace of purine in the chloragogen of *L. terrestris*.

Though these observations are interesting they cannot be the major concern when considering the excretory role of chloragogen since these are at best minor fractions of the urine produced, and may account for the so-called creatinine fraction of Bahl (1947b).

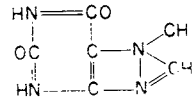

FIG. 19. Formula of heteroxanthine.

But urea and ammonia, as we have seen, are the most important excretory products, and these must be represented in the chloragogen if it is the site of origin of these substances.

In fresh chloragocytes obtained from *A. caliginosa* ammonia is found in large quantities, amounting to 0·60–1·36 μM/10 mg dry wt., whereas the urea content is negligible. After starvation the ammonia content is unaffected but urea now appears within the chloragogen at a level of 0·07–0·35 μM/10 mg dry wt. At the same time the nephridia hold 0·12–0·57 μM/10 mg dry wt., and the intestinal wall 0·12–0·18 μM/10 mg dry wt. (van Gansen, 1958). The transition to urea as a major excretory product after starvation has already been noted but the complete absence of urea from chloragocytes in fresh animals is not easily explained, although the amounts within the cells must be very small.

As mentioned previously, doubt has been cast upon the functioning of the Krebs–Henseleit cycle by Heidermanns (1937) as a result

of his work on the effects of amines on the nitrogenous metabolism of tissue homogenates. No information was available to him regarding the actual amines represented in the cells, but recently paper chromatographic studies have been made of the chloragogen cells. Cells isolated from fresh animals possess three amino-acids, ornithine, glutamic acid and occasionally arginine. Glycocol and kynurenine, a breakdown product of tryptophan in mammals, are also found. A period of starvation leads to the disappearance of ornithine, glutamic acid remaining and arginine being

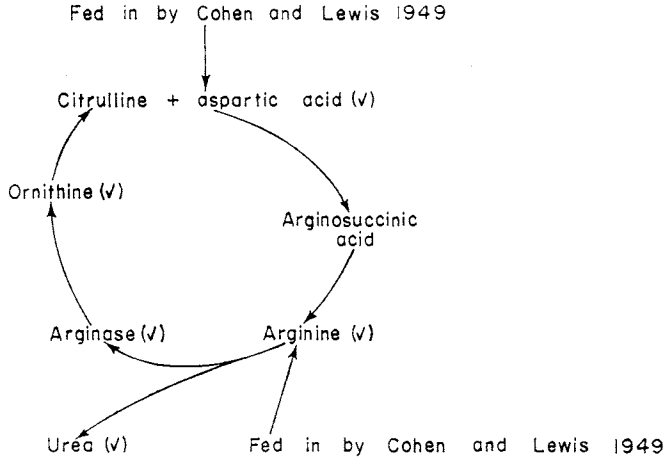

FIG. 20. Ornithine Cycle indicating components demonstrated as being present in or having an effect when fed to earthworms.

found now in all the animals studied instead of one or two. Citrulline is not present at either stage. The Krebs–Henseliet cycle of reactions (Fig. 20) involves the interaction of ornithine, arginine and citrulline. Glutamic acid is rapidly interchangeable with aspartic acid in mammals, but this reaction has not been shown in annelids. It also, by reason of combining with ammonia to form glutamine, can act as a carrier for this toxic waste product.

It can thus be seen that part of the cycle is present, but the absence of citrulline is confusing, especially so since Cohen and Lewis (1949b) report that the urea production of the earthworm

rises when this amine is injected into the alimentary canal, and rises further still when another nitrogenous compound is injected simultaneously. Arginine and alanine injected into the alimentary canal can both increase the excretion of urea (Abdel-Fattah, 1955). The only enzyme yet demonstrated in this cycle is arginase (Cohen and Lewis, 1950), but it is possible that citrulline is degraded very rapidly and thus is always at a minimal level in the cells, accounting for its non-demonstration. On the other hand some completely unexpected detoxication mechanism may be in force, and Needham (1960) suggests that the cycle may be halted at the ornithine stage, thus accounting for the lack of citrulline. The actual role of the chloragogen cell in the nitrogenous metabolism of the earthworm has recently been questioned in work done by Needham (1962). The part played by the enzyme arginase in the Krebs–Henseleit system has been mentioned above, and it has been shown to occur in extracts of chloragogen cells. The results of Needham (1962) indicate that the occurrence of arginase activity along the body of the earthworm is the mirror image of the presence of chloragogen, and it seems possible that this enzyme is associated with the gut wall.

From the observation discussed above it can be reasoned that the chloragogen cell is indeed liver-like (Roots, 1960). It is evidently involved in storage and metabolism as shown by its vast glycogen reserve and fat store, the concentration of acid ATP'ase indicates that it is a highly active tissue, and the concentration and variation of ammonia and urea content shows that it is taking part in detoxication mechanisms. It would be interesting to know the effects of removal of the chloragogen tissue on an oligochaete.

Removal of waste Products

If then, as it seems likely from the analyses above, the ammonia, urea and other nitrogenous compounds of the urine are formed in the chloragogen cells, how are these substances transported to the organs of excretion, the nephridia that open through the body wall or into the gut in some species?

Estimations made of the nitrogenous substances of the coelomic fluid and of the blood show that in both these systems there is less ammonia and urea than is available in the excreted urine, but that creatinine (? other guanidine) is at a lower level in the urine.

Protein is at a very much reduced level in the urine, and a great deal of what is present probably arises as the mucus secretion of the body wall (Table 9). It is, therefore, obvious that a filtering process is going on somewhere in the body.

Waste materials can arrive at the nephridia by one or more of three methods: via the blood system to the walls of the excretory organs, by the chloragogen cells that disintegrate or via the coelomic fluid. The disengagement of chloragogen cells from the surface of the alimentary canal has been described by Cuénot (1898) and by Liebmann (1946); the cells fall into the coelom and there disintegrate to release the contents into the fluid of the coelom, but Abdel-Fattah (1955) does not think that such actions occur. Earthworms allowed to feed upon a mixture containing iron saccharate absorb it into the alimentary canal walls, and it is also traceable in the chloragogen cells, but in no case free or in coelomic corpuscles. It is thus unproven that the chloragogen does indeed break down in the coelom, and in so doing releases excretory products for removal via the nephridia.

On the other hand, urea and ammonia are formed in the chloragogen, and find their way somehow into the coelom. This may occur by diffusion from the chloragogen, or by filtration from the blood system. The latter idea would require some special mechanism for allowing the passage of only nitrogenous materials. Only the blood contains large amounts of glucose, amino-acids and fats, but both blood and coelomic fluid contain ammonia and urea in similar quantities (Bahl, 1947). Abdel-Fattah (1955) believes that the blood system is the transport system for the waste products of tissue metabolism and filtration occurs at the nephridia. This would seem to be the obvious method in oligochaetes with internally closed nephridia e.g. *Megascolex caeruleus* or *Hoplochaetella*, but in species like *Lumbricus* the possibility of the coelomic contents being removed down the lumen of the nephridia by the beating of the nephrostome cannot be completely discounted, particularly since the chemicals are in solution in the coelom and are not contained within the cellular entities.

The Function of the Nephridia in Excretion

The nephridia are arranged segmentally within the oligochaete body and good descriptions of the various modifications and

structures are available from Bahl (1947), Goodrich (1945) and Stephenson (1930). See also the section on water relations and the work of Ramsay (1947, 1949).

Basically the nephridium is a tube, often open at both ends, internally and externally, and lined with cilia in tracts along its length. There is a good blood supply to this organ. The tube is specialized into distinct regions and the function of these regions is only partially known.

The many ciliary tracts that line the tubular nephridium are continually beating such that fluid passes along the lumen from the internal end to the external end. In those nephridia that are closed internally the tubular contents must obviously arise by filtration through the walls. In species having nephridia opening internally the contents may arise equally or unequally from the coelom and blood supply. If the majority of the fluid comes from the coelom then the chemical composition must be changed considerably during its passage along the tube. If it originates from the blood then many of the larger molecular substances such as protein, amines and organic food substances may be precluded from passing through the nephridial wall and thus never appear in the urine at all. Electrolytes pass through the walls into the lumen in both situations.

It is known that the composition of the urine changes as the fluid passes along the nephridium, Ramsay (1949) showing that the osmotic pressure changes are mainly a function of the "wide tube" (see Chapter VI). No work has been published to show whether salt resorption or water secretion occurs at this stage, although the phenomenon of granular resorption has been described by Cordier (1934). He found that small granules, less than 1000Å can be removed directly to the exterior via the nephridium. This compares with an upper size limit of 25Å passed by vertebrate kidney nephrons. Some granules in the earthworm, however, are accumulated by the ciliated middle tube wall, and the granules thus taken up are thought to be permanent fixtures in this situation until the end of life (Stephenson, 1930; Bahl, 1947b). The middle tube of the nephridium thus seems to act as a kidney of accumulation. It was suggested by Willem and Minne (1900) that the naturally occurring granules observed in this situation are guanine, in common with the granules of chlora-

gocytes according to these authors, but this has been discounted by Bahl (1947b), who also disposed of the idea that they are uric acid. Guanine is not soluble in ammonia or acetic acid or dilute alkali, but the granules of the nephridium are, and they also dissolve to give a coloured solution in pyridine. This solution has absorption bands at 558 mμ (α) and 527 mμ (β), and Bahl suggests that this is a haemochromogen deriving as a breakdown product from the blood. Further work may clarify this point since protoporphyrin (Dhéré, 1932; Laverack, 1960a) is known to be laid down in the body wall during life, giving characteristic iridescence to the integument, and this substance is soluble in pyridine. The absorption peaks differ from those of the nephridial granules. Roots (1960) has recently investigated the brownish-yellow pigment of the chloragogen cells and found that this may be a complex arrangement of substances which are soluble in ammonia and pyridine, but possess no distinct absorption spectrum. This may bear some resemblances to the nephridial material, but the question needs further work.

From his comparative studies on the blood, coelom and urine of *P. posthuma*, Bahl (1947b) concludes that nephridia carry out three functions in earthworm excretion, namely filtration, re-absorption and chemical transformation. Filtration, in the sense of pouring a liquid through a coarse sieve, but not ultrafiltration under pressure through a membrane as in the vertebrate kidney, occurs at the nephrostome. Thus in nephridia open to the coelom, protein passes into the lumen of the nephridium, and it is unlikely that it can do so from the blood vessels by ultrafiltration since the pressure within these vessels is low (*ca.* 4·4 mm Hg at rest and >9·3 mm Hg when active, Prosser *et al.*, 1950). As the protein appearing in the urine is far less than that in the blood and coelomic fluid, and much of what is present is accounted for by the secretions of the body wall, it follows that much active re-absorption of protein occurs through the nephridial wall against a concentration gradient. Electrolytes such as chloride, sodium, potassium and phosphate all diminish in quantity as they pass along the tubules since the final concentration is lower in the urine than in the coelomic fluid and blood. Although the evidence is not conclusive, Ramsay's observation that urine becomes hypotonic in the wide tube, and possibly more so in the middle tube is also suggestive of

the re-absorption of substances in these regions. Martin (1957) recently discussed these results in the light of more modern knowledge in other invertebrates. He considers that if blood is filtered across the closed nephridium of *Pheretima* a large osmotic inflow of water would occur, thus forming a hypotonic urine, and causing the animal to handle up to 50% of the body volume of water in 24 hours. Bahl (1947) found a urine production of very nearly this figure (45%) in animals maintained in a water-saturated atmosphere, equivalent to an excretion of 0·3–0·4 ml/hr/worm. Chapman (1958) considers that a urine production rate very similar to this is shown by *Lumbricus* as well. If this is so then the hypotonic production may be due to flooding of the nephridium. Electrolytes may be actively re-absorbed through the nephridial wall, passing into the coelom (or blood?), and after the elapse of some further period isotonicity would again be established between coelom and blood, (Fig. 21).

Alternatively Bahl (1947b) indicates that filtration from the blood capillaries surrounding the nephridium may occur, even in situations where they are open to the coelom. This may be facilitated by the beating of the cilia within the tubules. A slight pressure would be set up in the tubule and this may act in drawing liquid through the walls of the nephridium. Carter (1940) previously considered this to be unlikely as the colloid osmotic pressure of the fluid bathing the cells is too great, but Pantin (1947) has shown that the flame cell activity of a nemertine *Geonemertes dendyi* increases as fluid passes into the animal and osmotic regulatory mechanisms come into play. This sort of action may occur in annelids as well. Indeed Roots (1955) reports that after bathing with hypotonic fluids the cilia of the nephrostome (*Lumbricus terrestris* and *Allolobophora chlorotica*) undergo a period of increased activity in rate of beat and sometimes amplitude as well. This is followed by a return to normal. In cases of hypertonicity the ciliary beat slows.

The question of re-absorption of materials from the nephridium Martin (1957) considered to be unconfirmed, particularly the removal of protein from the fluid in the lumen. He considered that the differences in blood and urine levels of protein, which are in the ratio 16:1 requires that the coelom should provide more than one-sixteenth of the urine filtered. Another objection is that, in

Pheretima at least, the nephridia drain, not to the outside, but into the gut and the absorption of protein may occur here rather than

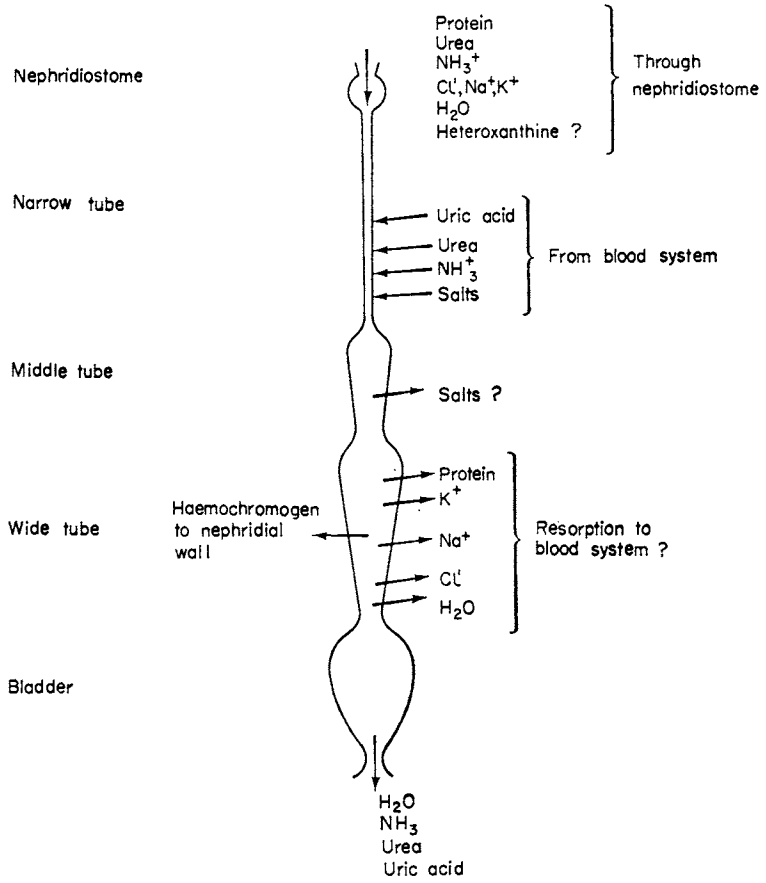

FIG. 21. Diagram to summarize possible mode of functioning of oligochaete nephridia (*Lumbricus*).

in the nephridium. Ramsay (1949b) found that whilst a considerable coagulation occurs upon heating blood from *Lumbricus* only a very slight coagulum occurs with coleomic fluid or urine, and as urine probably derives from both sources in this species the protein

content of the final fluid can be expected to be less than that of the coelomic fluid.

Summary

Nitrogen is lost in the form of mucus secreted by the epidermis and in urine.

Earthworms excrete ammonia, urea and also, possibly, uric acid, allantoin and doubtfully creatinine. The concentrations of these substances varies according to the diet. In animals under normal conditions of food and water intake, ammonia is the dominant constituent in urine with smaller quantities of urea; under conditions of starvation the position is reversed, urea increasing above the previous level and above that of ammonia. Some evidence is available that the chloragogen cell is involved in excretion probably via the Krebs–Henseleit cycle of reactions. The enzyme arginase, and the amino-acids ornithine, glutamic acid and arginine have been shown to be present but not citrulline. Heteroxanthine may be present in chloragogen. There are indications that re-absorption of material occurs in the nephridia but filtration from the blood and coelom may be efficient enough to account for differences in analyses of urine, blood and coelomic fluid.